Studies in Biohythane Production from Petha Waste

Synopsis of the work proposed for the
Partial fulfilment of Ph.D. degree in Chemistry

Submitted by

POOJA CHAHAR

Forwarded

Prof. Sahab Dass
Head

Dr. Radhika Singh
Supervisor

Prof. Ravinder Kumar
Dean

Dept. of Chemistry
Dayalbagh Educational Institute
Dayalbagh, Agra-282 005
September 23, 2016

1. Introduction

Fossil resources are not sustainable and are questionable from economic, ecological and environmental point of views (Kamm et al., 2006). The burning of fossil fuels has significant contribution to the increasing level of CO_2 in the atmosphere, which is directly associated with global warming, observed in recent decades (www.shell.com). The adverse effect of greenhouse gases (GHG) emission on the environment and declining petroleum reserves with exponential energy demand has been noticed (Mabee et al., 2005). The excessive usage of fossilized fuels in various industries, automobiles and other sectors has led to its depletion, forcing the mankind to look for alternative fuels like solar, wind, hydro power, thermal, etc. (Noori and Saady, 2013; Nagendrantha Reddy et al., 2015). The current rate of consumption of petroleum and coal and the exhaustion of these reserves thus necessitated the search for an alternative source of energy (Soetaert and Vandamme, 2009). It has triggered focus on renewable and development of greener technologies to fulfill the growing energy demands (Das and Verziroglu, 2001). The linear system is converted to a circular one and turning waste into a resource is part of "closing the loop" and deriving energy from waste is a valuable way to fulfill this objective as well as reducing global fossil fuel dependence (Bilitewski, 2012). Different strategies like thermo chemical conversion (liquification/pyrolysis/gasification/combustion), biological conversion (anaerobic digestion/fermentation/enzymes), chemical conversion (hydrolysis/solvent extraction/supercritical conversion of biomass) and physical conversion (mechanical extraction/distillation/briquetting of biomass) could be applied to recover energy from waste (Naik et al., 2010). Biomass derived from trees, agro-forest residues, grasses, plants, aquatic plants and crops are versatile and important renewable feed stock for chemical industry as shown in Fig 1.

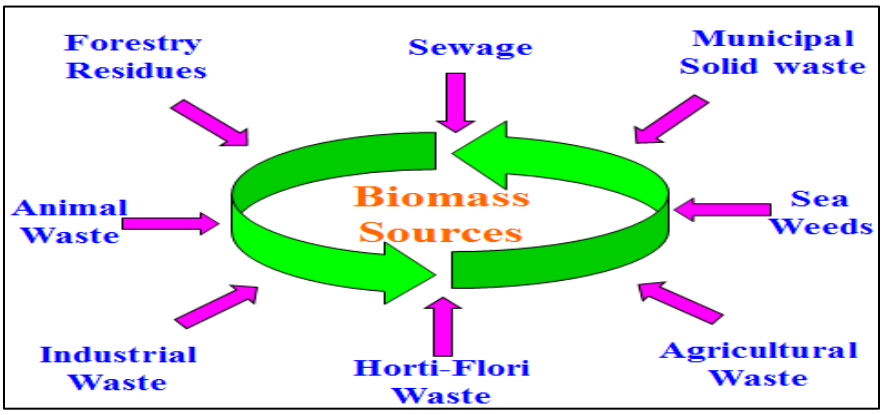


Fig 1 Biomass Sources (Source: Heinimo and Junginger, 2009)

Anaerobic Digestion (A.D) is a widespread technology for waste treatment and biogas production (Baere et al., 2010). AD is a series of biological processes that use a diverse population of bacteria to break down organic material into biogas, primarily methane and combination of other gases (like carbon dioxide, hydrogen etc.); it occurs in the absence of free oxygen (Antoni et al., 2007). AD process occurs in four interacting steps: (Bharathiraja et al., 2014)

Step1 Hydrolysis: Long chain polymers like carbohydrates, lipids and proteins are hydrolyzed to short chain monomers and dimers like fatty acids, amino acids and sugars.

Step2 Acidogenesis: The monomers and dimers from step1 are turned into organic acids (e.g. propionic acid, butyric acids, and valeric acids). During this process step hydrogen, carbon dioxide and alcohols are also formed.

Step3 Acetogenesis: During the acetogenesis process step, acetic acid (CH_3COOH), carbon dioxide, and hydrogen are formed.

Step4 Methanogenesis: During methanogenesis, acetate is converted into methane and CO_2, whole hydrogen is consumed.

Feedstock characteristics are important factors affecting biogas production and process stability during anaerobic digestion. The main characteristics of feed stocks include moisture content, total

solid (TS), volatile solids (VS), particle size, pH, biodegradability, chemical oxygen demand (COD), biological oxygen demand (BOD), carbon and nitrogen ratio (wikipedia: Anaerobic digestion). Typical concentration of compounds in biogas is summarized in Table 1.

Table 1 Volume percentage ranges of compounds contained in biogas (Source: http//wwwseilnach.com/lexikon/ebiogas.html)

Compound	Formula	Volume (%age)
Methane	CH_4	40-75
Carbon dioxide	CO_2	20-55
Water vapor	H_2O	0-10
Nitrogen	N_2	0-5
Oxygen	O_2	0-2
Hydrogen	H_2	0-1
Ammonia	NH_3	0-1
Hydrogen sulfide	H_2S	0-1

AD of biowaste can also be carried out in a two phase thermophillic AD process for the co-production of hydrogen from the first phase anaerobic reactor and methane from the second-phase reactor. The production gas mixture is called Biohythane (Porpatham et al., 2007). It was demonstrated that using this enriched biogas; engine performance could be improved taking into account the complementarity of hydrogen and methane (Porpatham et al., 2007; Cavinato et al., 2011). Hydrogen has emerged as a promising alternative fuel since it does not produce CO_2 on combustion; it also has the highest calorific value and high combustion velocity (release of H and OH radical). The typical composition of biohythane is 5% to 10% hydrogen, 30% to 40% carbon dioxide and 50% to 65% methane (Chinellato et al., 2013). A very promising approach embracing all the above requirements is the combined biological production of biohydrogen and biomethane, which can be performed through a two-stage biological fermentative process by anaerobic dark fermentation of carbohydrate based wastes in the first-stage and anaerobic digestion of the product of first stage in the second-stage (Dounavis et al., 2015). The biohythane production process, shown in Fig.2, comprises of biohydrogen production followed by biomethanation. The total gaseous energy recovery for two stage

process was found to be 53.6%. While gaseous energy recovery from single stage was only 28% from single stage. Thus, two stage systems not only help in improving gaseous energy recovery but also can make biohythane (mixture of hydrogen and methane) concept commercially feasible (Roy and Das, 2016). Biohythane (Table 2) is more effective than primary fuel because of greatly reduced NO_x emission and several hydrocarbon emissions. The combination of hydrogen and methane as vehicular fuel proposes several advantages such as (Moreno et al., 2012):

- Methane has lower fuel efficiency due to its lower flammability. Lean flammability range of methane could be improved by addition of hydrogen.
- Hydrogen has eight-fold more flame speed as compared to methane in lean air/fuel mixture.
- For accelerating the methane combustion, use of hydrogen could stimulate a powerful combustion within an engine. Hydrogen is also a powerful reducing agent for the efficient catalysis at lower exhaust temperatures.

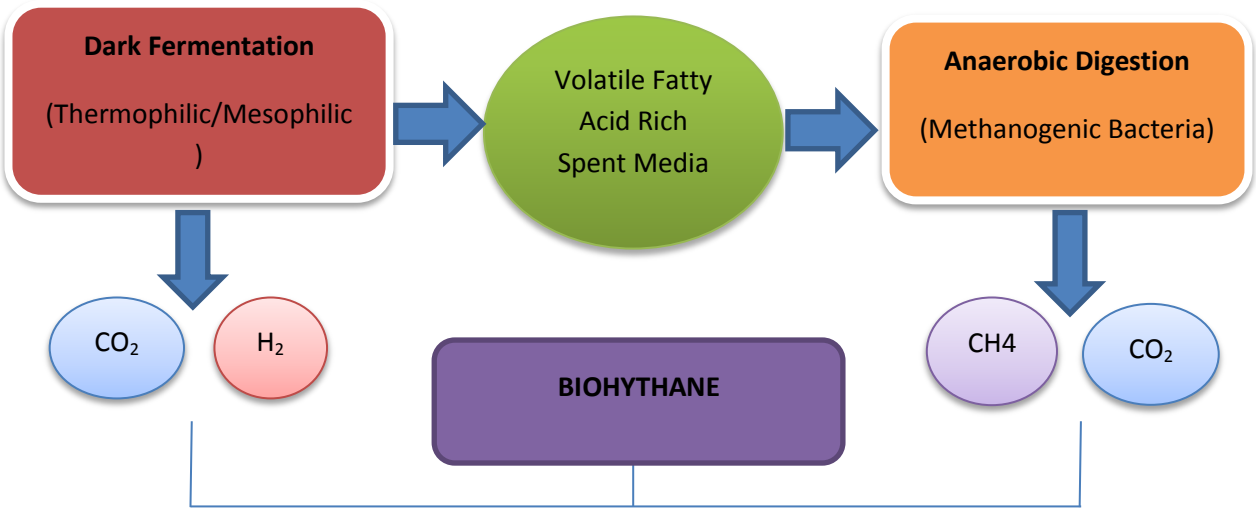


Fig2. Systematic production of biohythane from organic waste by dark fermentation and anaerobic process (Source: Roy and Das, 2016)

Table 2 Properties of biohythane (Source: Klell et al., 2012)

Property	Unit	Methane	Hydrogen
Molar mass	kg/Mol	16.043	2.016
Density (at 1 bar, 25°C)	kg/m^3	0.648	0.081
Calorific value	kJ/kg	50,020	119,930
Ignition limit (in air at 1 bar , 25°C)	Vol %	4.4-1.5	4-16
Auto ignition temperature (in air at 1 bar)	°C	595	585
Laminar flame speed (in air at 1 bar, 25°C,lamda=1)	cm/s	42	230

pH is one of the most important parameters that affects the metabolic pathway of hydrogen production in which the glycolytic and supportive enzymes (Fe-Fe hydrogenase, formate lyase etc.) are the key players. During the course of dark fermentation, the pH profile changes due to the accumulation of metabolites like volatile fatty acids. The accumulation of volatile fatty acids is detrimental for the microorganisms as the cell membrane's integrity is destroyed, which eventually leads to disruption of maintenance of internal pH (Khanal et al., 2004). Methanogens or hydrogen consuming microbes are suppressed at low pH. In the controlled pH ranges of 5.5 to 6.5, a significant improvement in hydrogen production was observed with highest hydrogen yield of 1.72 mol/mol of xylose at a pH of 6.5 (Calli et al., 2008). Microbes responsible for hydrogen and methane production have been found in varied temperature ranges. Most of the literature reports on biohydrogen and biomethane production are based on mesophilic dark fermentation (Li et al., 2001). For hydrogen production, temperature plays a vital role (Hung et al., 2011**;** Roy et al., 2014). If the temperature is increased by a factor of one degree celsius, the enzymatic activity increases by factor of two. Fermentative microorganisms capable of producing hydrogen were reported at different temperatures: pscycrophilic (0°C to 20°C), mesophilic (20°C to 42°C), and thermophillic (42°C to 75°C). Hydrogen production increased with increase in temperature from 30°C to 40°C. On further increasing the operational temperature, there was decrease in hydrogen production (Fabiano and Perego, 2002). The

partial pressure of hydrogen inside the reactor increases as it starts accumulating in the head space. Decreasing partial pressure by intermittent nitrogen sparging helped in improving the hydrogen yield by 68% (Mizuno et al., 2001). Hydraulic retention time (HRT) is other important factor that affects the hydrogen producers and methanogens. On working with mixed consortia, which contain methanogens and acidogenic hydrogen producers, manipulating the HRT might lead to shift in microbial profile inside the reactor. Changes in HRT might help in expelling slow growing microbes from the reactor system. Methanogens and other hydrogen consuming microbes are slow growing microorganisms (Zhang et al., 2006). Thus, shorter HRTs help in selective enrichment of hydrogen producing microbes. On lowering HRT from 10 hours to 6 hours, the hydrogen production by *Clostridium* sp. was found to increase, which was followed by decrease in propionate production (Zhang et al., 2006). On using anaerobic sludge, HRT of 1 day showed higher hydrogen production and increased butyrate/acetate ratio (Mariakakis et al., 2012). No methane was detected in the biogas. Inoculum is another very important factor for achieving desired product. Mixed microbial consortium is symbiotically associated with different groups of bacteria. The natural microbial flora consists of different types of microbes such as hydrogen producing bacteria, hydrogen consuming bacteria or methanogenic microorganisms, and acetoclastic electrogens. A single group of bacteria might not have all the hydrolytic enzymes required for hydrolysis of complex organic compounds like cellulose, xylan and lignin. Hydrogen producing microorganisms are present in various natural and manmade habitats. They are found in prominence in sewage sludge, anaerobically digested sludge, acclimated sludge, compost, animal manure, hot springs, oceanic sediments, and soil (Chen et al., 2005; Sparling et al., 1997; Ueno et al., 1995; Lin and Lay, 2005). The major drawback of mixed consortia is the chance of dominance of non-hydrogen producing microorganisms, methanogens or hydrogen consuming microorganisms and lactic acid producing bacteria (Roy and Das, 2016).

Advantages of biohythane (Kasianantham et al., 2011)

- ✓ Biohythane is easily usable with the existing infrastructure.
- ✓ It requires only small amount of hydrogen storage and a column for the mixing of hydrogen with the natural gas.
- ✓ It is safer to handle than hydrogen, because of lower risk due to very low energy content from hydrogen.
- ✓ It extends the lean misfire limit of CNG.
- ✓ Hydrogen addition to natural gas can decrease engine's unburned hydrocarbons and NOx emissions and speed up the combustion process.
- ✓ It improves the engine efficiency and lowers fuel consumption.

Comparative properties of Hythane, hydrogen and methane are shown in table 3. HCNG (5) means the Hythane consist of 5% hydrogen and 95% methane.

Table 3 Comparison of properties of hydrogen, methane and HCNG (5)
(Source: Kavathekar et al., 2007)

Properties	Hydrogen	HCNG (5)	Methane
Limits of flammability in air (vol. %)	4-75	5-35	5-15
Flame temperature in air (K)	2318	2210	2148
Auto ignition temperature (K)	858	825	813
Minimum energy for ignition in air (mJ)	0.02	0.21	0.29
Stoichiometric composition in air (vol. %)	29.53	22.8	9.48
Methane number	0	76	80

***HCNG: hydrogen + compressed natural gas where 5 indicates volume percentage of hydrogen.**

2. Review of literature

Sequential dark H_2, photo H_2 and CH_4 fermentation of cassava ethanol waste water for optimum hydrogen production and energy yield was carried out by Lin et al. (2016). Treated solution of dark

H_2 (with zeolite) added to photo hydrogen fermentation resulted in maximum hydrogen yield. Hydrogen yield was 369.7 mL/g CODs and methane yield was 117.9 mL/g CODs. Co-generation of hydrogen and methane gave an energy yield of 8.39 kJ/g CODs.

Production of biohythane from giant reed, maize, olive pomace and wheat bran from combined system (dark fermentation + anaerobic digestion) was evaluated by Corneli et al. (2016). Batch test was done under optimum conditions (at mesophilic temperature, without pH control). In order to study the effects of dark fermentation on estimated energy recovery in the combined system, single step anaerobic digestion of substrates was also done. Maximum biohythane potential and energy recovery was obtained in the combined system with wheat bran as substrate.

Kumari et al. (2016) observed maximum energy recovery from pretreated sugarcane top using biohythane process. The maximum hydrogen production yield was 87.40 mL/g-VS and highest methane yield was180.86 mL/g-VS. Recovery of total gas energy was 37.7%, which was 54% more than that of untreated sugarcane.

Synergistic effect for pro-culture of extreme thermophiles for optimum biohydrogen dark fermentation xylose, cellobiose and garden waste were studied by Angela et al. (2016). Co-culture of C. *Saccharolyticus* and *Caldicellulosiruptor bescii* yielded higher hydrogen from xylose (2.7±0.1) and cellobiose (4.8±0.3)) as compared to individual culture. Higher hydrogen production yield from garden waste obtained from co-culture of extreme thermophiles (C. *Saccharolyticus* and *Caldicellulosiruptor bescii)* as compared to individual culture of xylose and cellobiose. Biogas enriched with 15% hydrogen (biohythane) with a potential energy generation of 22.2 MJ/kg-VS was obtained from garden waste.

Sarkar et al. (2016) used orthogonal array to design optimized experiments to evaluate the influence of eight critical factors on multiple metabolic output parameters. Enhanced biogas and volatile fatty

acids production was observed under a specific regime of selected factors whereas, pretreated inoculum and high substrate concentration increased hydrogen and volatile fatty acids production. Ratio of H_2/H_2+CH_4 varied from 0.1 to 0.97 and use of untreated inoculum enhanced biohythane production.

Elreedy et al. (2015) investigated biohythane production from petrochemical wastewater and the reactor was continuously operated for five months at constant hydraulic retention time (HRT) of 72 hour with different organic loading rates (OLRs) of 0.33, 0.67 and 1.67 g COD/L-d. The maximum hydrogen yield was 59.01±33.46 mL H_2/g-COD removed and the maximum methane yield was 159.11±14.72 mL CH_4 /g-COD removed. The maximum methane and hydrogen content of 52.08% and 49.84% were achieved at OLR of 0.33 and 1.67 g COD/L-d, respectively.

A two stage biohythane system comprising of up flow anaerobic sludge blanket reactor and packed bed reactor were operated and COD removal and energy recovery was found to be more than in one stage biomethane system (Si et al., 2015). The first stage of hydrogen fermentation had a positive effect on following biomethane production in the two stage biohythane system. In comparison to the biomethane system, the relative abundance of acidogens like *Clostridiaceae* was reduced and acetogens like *Synthrophaceae* and acetate oxidizing bacteria like *Spirochaetes* were increased in the biohythane system.

Dounavis et al. (2015) have produced biohydrogen enriched methane from waste glycerol in a two stage continuous systems. An attached mixed acidogenic consortia was used in an up flow bioreactor in the first stage to study biohydrogen production. The effluent of this reactor was fed to the second stage methanogenic reactor, which was a continuous stirred tank reactor. Product gases of both reactors (stage I and stage II) were mixed to form biohythane (fixed biofuel). With 20 g/L glycerol, hydrogen was produced with a yield of 0.051, 0.070 and 0.094 L/g-COD_{fed} with feed pH values 6, 6.5

and 7 respectively. Methane produced in the second stage was with a yield of 0.257 L/g- COD_{fed} with commercial glycerol in the feed. When crude glycerol was taken, methane production was 0.283 L/g-COD_{fed}. When effluent of the first stage reactor was diluted to 5, 7.5 and 10 g COD/ L, methane production was 0.198, 0.242 and 0.273 L/g-COD_{fed} respectively.

Gottardo et al. (2015) have reported biohythane production by two phase codigestion from biowaste and sewage sludge. Biohydrogen was produced 40 L/kg-TVS_{fed} in the form of specific hydrogen production and 320 L/kg-TVS_{fed} specific methane production was noticed in the second phase. The biogas (biohythane) produced had a composition (methane 58.6 %, hydrogen 7.5 % and carbon dioxide 33.9 %).

Production of biohythane from marine macro algae *Sargassum sp.* was evaluated by Costa et al. (2015). Observed yield of hydrogen was 91.3±3.3 L H_2/kg-VS of *Sargassum sp.* and methane yield was 541±10 L H_2/kg-VS. Total energy production estimated in two stage systems was 242 G J/ha yr and maximum energy of 600 EJ/yr is possible form the ocean potential area for microalgae production.

Micolucci et al. (2014) studied automatic control process for biohythane production from food waste by two phase anaerobic digestion. Under managed conditions (variable recirculation flow) a mixture of gases was produced with an average composition range of 7% hydrogen, 58% methane, and 35% carbon dioxide. Observed overall specific gas production and gas production rate were 0.69 $m^3_{\mathbf{Biogas}}$/kg-TVS and 2.78 $m^3/m^3_r d$.

Pisutpaisal et al. (2014) evaluated the total energy recovery from food waste fermentation (at first stage hydrogen, and at the second stage methane were produced) in a two-stage 5-L CSTR system, under mesophilic temperature with the initial pH 6 and 7; and hydraulic retention time of 12 h and 24 h, respectively. The results showed that the hydrogen and methane yields were 292.7 and 391.6 mL/g-

VS at the steady state operation. The methane yields in the one-stage from food waste fermentation were 364.3 mL/g-VS. The total energy recovery from two-stage process was $6.5x10^{-2}$ kW/h, while that from one-stage process was $4.7x10^{-2}$ kW/h.

Waste milk (WM) was produced by sick cows undergoing antibiotic treatment (Suraju et al., 2014). This WM was codigested anaerobically along with cow manure (CM) in a two stage process at 55°C (thermophilic range). Hydrogen and methane potentials, volatile solids (VS) removed and energy recovery was studied. Lag phases to hydrogen and methane production were observed in WM: CM mixtures, which increased with increase in the ratio. The sytems failed with 90% WM in the feed. Optimum ratio found was WM:CM 70:30, but this was not economical because of lag phase observed and limited amount of waste milk available. WM:CM ratio of 30:70 was found to be the minimum limit for significant increase in hydrogen and methane yield and was recommended in practice.

Production of biohythane in a two-phase anaerobic digestion process treating food waste as substrate was investigated by Chinellato et al. (2013). They carried out preliminary batch assays at initial organic loadings of 15, 20, 25 and 30 kg- TVS m^3 in stirred reactors (1.5 L) at 55°C. The results showed that within the first 24 hour, all hydrogen was produced after feeding and the highest load tested gave the maximum hydrogen production (0.047 m^3H_2/kg-VS, 30%). Similar loadings were then tested in a two-phase system. HRT of 3 and 12 days were applied to the first and second reactor respectively. In order to keep the pH at 5.5, either supernatant or whole digestate from the methanogenic reactor was recirculated to the first phase. Results showed that hydrogen was produced (0.117 Nm^3/kg-VS, 47.7%) when recirculating whole digestate with an organic loading rate of 20 kg-TVS/m^3/day.

Kvesitadze et al. (2012) have reported cost-effective two-stage anaerobic fermentation process (bio-methane and biohydrogen). In natural environments, hydrogen producing anaerobes coexist with

methane forming ones which consume intermediate products of hydrogen fermentation including hydrogen and produce their own end products like methane and CO_2. An obstacle to obtain hydrogen as the final product of dark fermentation is associated with partitioning of hydrogen and methane fermentation processes in cost effective way. The use of 10% solution of sodium hydroxide completely inhibited methane forming bacteria. Under thermophilic conditions and pH 9.0, cumulative bio-hydrogen production was 82.5 L/kg dry organic matter. 50.1% of hydrogen and 49.8% carbon dioxide were produced. Content of methane in biohydrogen did not exceed 0.1 %. Addition of inoculum of hydrogen producing bacteria promoted the increase of biohydrogen cumulative production up to 104 L/kg dry organic matter. Cumulative bio-methane produced from the metabolite products of hydrogen fermentation made 520 L/kg dry organic matter. Methane and carbon dioxide percentages were 78.6% and 21.4% respectively. Compared energy data for two stage anaerobic digestion with those for single stage process, resulting solely in bio-methane production shows that energy output from biodegradable fraction of MSW Increases by 23% to 26% in two stage anaerobic process.

Lustrato et al. (2012) have reported production of methane and hydrogen via both conventional mono and high-rate two-phase anaerobic digestion processes from large distribution organization waste vegetable residues. At first, in order to set up and optimize several parameters related to the increase both in bio-hydrogen and methane, several aspects were considered, under lab conditions such as temperature, type of substrate, pre-treatments of solid wastes, type of microbial inocula, HRT, biofuels recovery etc. Two anaerobic reactors were connected in series for the experiment with working volumes of 1 L and 5 L for biohydrogen and methane, respectively. The system operated in semi-continuous flow, at pH 5.5 and a HRT of 4 day, while the methane stage at pH 6.5-7.5 and a HRT value of 16 day.

Cavinato et al. (2012) investigated the optimal process operational conditions of thermophilic dark fermentation and anaerobic digestion of food waste. They also here reported the effect of HRT and pH on hydrogen production where they applied an OLR of 16.3 kg-TVS/m^3 d in the first phase and 4.8 kg-TVS/m^3 d in the second phase. First and second phase hydraulic retention times were maintained at 3.3 days and 12.6 days. pH was maintained between 5 and 6. Specific hydrogen production was 66.7 L/kg-TVS fed and a specific biogas production in the second phase was 0.72 m^3/kg-TVS fed in long term test run. They obtained hydrogen (6%) and methane (58%) in the gas mixture respectively.

Peixoto et al. (2012) discussed the potential for hydrogen and methane production by using different effluents in two step batch fermentation process. 23.9 mL/g-COD hydrogen was produced from parboiled rice waste water and from vinasse (by product of sugarcane) produced hydrogen was 20.8 mL/g-COD. Maximum methane was produced from vinasse (255.4 mL/g-COD) whereas in parboiled rice waste water methane produced 115.5 mL/g-COD. Corresponding to the maximum COD reduction, total energy recovery from vinasse (10.4 kJ/g-COD) was 74.7%. Organic rich effluents have been used for energy recovery with generation of hydrogen and methane.

Cavinato et al., (2011) studied the production of biohythane from municipal solid waste by two-phase thermophilic process. Experiment was done at pilot scale using two stirred reactors of working volume 0.20 m^3 and 0.76 m^3 maintaining thermophilic condition (55°C). The experiment was done in three runs where HRT was maintained 3d for first and 12d for second reactor and OLR was changed (16, 21, 14 kg-TVS/m^3d) respectively. Yield of hydrogen in term of specific hydrogen production was 74 L/kg-TVS_{fed} (40% of hydrogen in first phase biogas) and biohythane was 0.98 m^3/kg-TVS_{fed} (specific gas production) respectively.

3. Motivation for present study

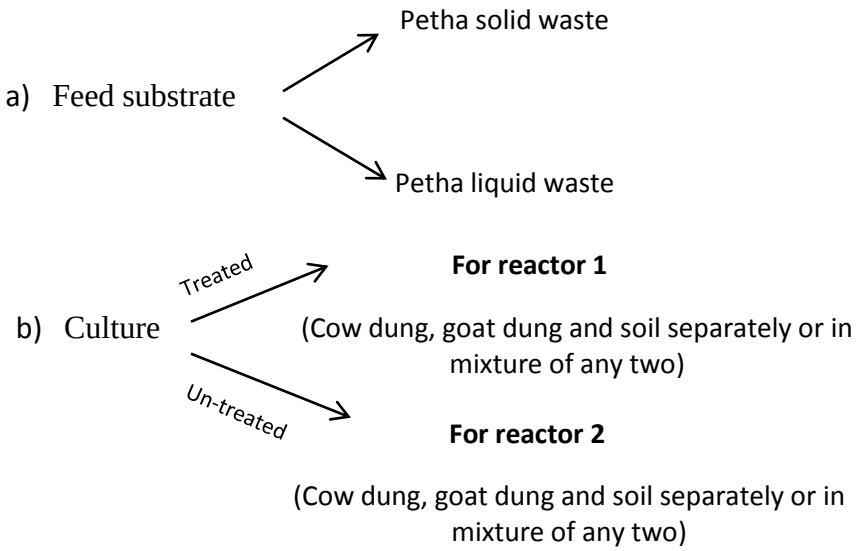
Agra is known for the Tajmahal and its petha sweet which is prepared from petha (*Benincasa hispida)* fruit. There are about 600 petha sweet producing small industries in and around Agra and produces a huge amount of alkaline (pH of 13-14) petha waste water and solid petha waste. These industries discard untreated petha solid and liquid waste into the river steams which disturbs the nearby aquatic-ecosystems (Singhal and Singh, 2015). The present research proposal will provide a solution to petha waste and its waste water by extracting clean and green energy (in the form of biohythane) from this waste. This would solve the two pronged problems: firstly, the problems caused by the unorganized disposal of the petha waste and secondly the energy crisis by producing clean vehicular fuel i.e. biohythane from petha waste through this scientific intervention.

4. Objectives and scope of present proposed research

The present research work is planned with the following objectives:

1. Optimization of ratio of petha solid waste and petha waste water for maximum production of hydrogen and soluble metabolite products during dark fermentation.
2. Pretreatment of inoculum using various physical methods viz. heat shock, pH, Microwave etc. for hydrogen production through dark fermentation methods.
3. Anaerobic degradation of spent effluent of dark fermentation process for optimum methane formation.
4. Optimization of process parameters for maximum production of biohythane from petha waste.
5. Carrying out statistical analysis of the obtained and modeling of the data.

5. Materials


a) Feed substrate
Petha solid waste
Petha liquid waste
b) Culture
Treated
Un-treated
For reactor 1
(Cow dung, goat dung and soil separately or in mixture of any two)
For reactor 2
(Cow dung, goat dung and soil separately or in mixture of any two)

6. Proposed methodology

1. **Preparing/pre-processing of feed substrate/feed stock:** Petha solid waste and petha waste water will be collected from the local petha processing industries and brought to the laboratory. Solid petha waste would be grinded to small pieces (slurry) and mixed with petha waste water in an appropriate ratio (50:50, 60:40 and 80:20 etc.). This mixture will be used as substrate in optimum ratio and its pH would be adjusted in between 6 to 7 for optimum hydrogen production.
2. **Characterization of feed substrate/feed stock:** Characterization of the substrate will be carried out by using APHA standard methods for solid waste and wastewater (APHA-AWWA, 1995). The main characteristics of feed stocks include moisture content, total solid (TS), volatile solids (VS), particle size, pH, biodegradability, chemical oxygen demand (COD), biological oxygen demand (BOD), carbon and nitrogen content will be observed using various methods and techniques.

3. **Preparation of culture for reactor 1:** Different samples of cow dung, goat dung and soil from specific sites will be collected and brought to the laboratory. Slurry of these samples will be used as microbial source for hydrogen production individually or mixed with one another to get a perfect combination of microorganisms. The best consortia would be further treated with various techniques like microwave, UV rays, heat socks etc. to get only hydrogen producing microorganisms for the first reactors. The developed consortia finally will be used as inoculum for hydrogen production from petha waste in reactor 1.
4. **Preparation of culture for reactor 2:** The above untreated samples (cow dung, goat dung and soil) were also used for the inoculum development in various ratios and developed into perfect methanogenic consortia by incubating and acclimating with substrate and finally used as inoculum for methane production in the reactor 2.
5. **Operation of reactor 1 for biohydrogen production:** Reactor 1 will be used for the production of bio-hydrogen. This bioreactor 1 would be operated under anaerobic conditions. Various process parameters like solid and liquid waste ratio, substrate and inoculum ratio, reactor charging rate, process temperature, pH, agitation rate, substrate digestion rate, gas production time etc. will be optimized for maximum production alcohol and volatile fatty acids in terms of acetate, butyrate, propionate and finally hydrogen production will be detected by gas chromatography.
6. **Operation of reactor 2 for methane production:** Reactor 2 would also be operated under anaerobic conditions for biomethanogenesis. Just like reactor one various process parameters like substrate and inoculum ratio, process temperature, pH, agitation rate, substrate digestion rate, gas production time etc. will be optimized for maximum

production of methane. The various gases CH_4 and CO_2 will be detected by a gas chromatography (GC) using a thermal conductivity detector (TCD).

7. **Blending of outputs of from reactor 1 and reactor 2 and their optimization to produce different kinds/blends of biohythane:** Biohydrogen produced in the reactor one and methane in reactor two will be collected and mixed in various ratios to get a perfect combination of biohythane.
8. **Analysis of data, results and discussion:** The data generated through this research work will be analyzed using special software's and results will be interpreted according to the findings and will be discussed with relevant justification along with already existing data and results present in scientific domain.

Schematic representation of biohythane formation from petha waste can be represented as-

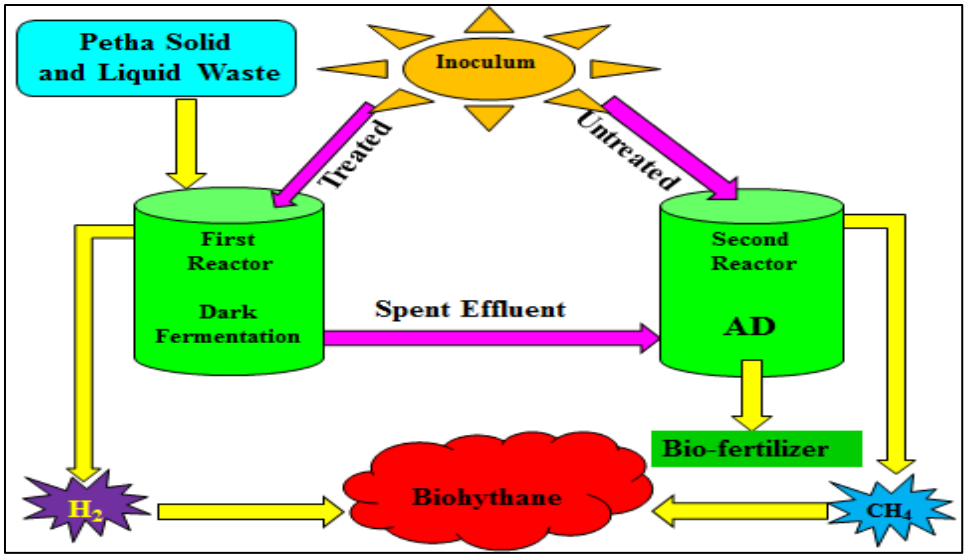


Fig. 3 Flowchart showing proposed methodology

7. Work plan

Time line	Research Work
Jan-June 2017	Characterization of waste and ratio optimization of petha solid waste and petha liquid waste (petha waste water).
July-Dec 2017	Effects of different types of inoculum on DF and AD process.
Jan-June 2018	Effects of pretreatment for the maximization of hydrogen yield.
July-Dec 2018	Optimization of methane yield during AD and Optimization of parameters for maximization of biohythane.
Jan-June 2019	Assembling of data.
July-Dec 2019	Results and data calculation by applying statistical methods.

References

Abreu AA, Tavares F, Alves MM, Pereira MA (2016), Boosting dark fermentation with co-culture of extreme thermophiles for biohythane production from garden waste. Bioresource Technology, 219:132-138.

Antoni D, Zverlov VV, Schwarz WH (2007), Biofuels from microbes. Applied Microbiology and Biotechnology, 77(1):23-35.

APHA (1995), Standard methods for the examination of water and wastewater. 20th ed. J, Washington, DC: American Public Health Association/ American Water Works association/ water Environment Federation,.

Atif AAY, Fakhru'l-Razi A, Ngan MA, Morimoto M, Iyuke SE, Veziroglu NT (2005), Fed batch production of hydrogen from palm oil mill effluent using anaerobic microflora. International Journal of Hydrogen Energy, 30:1393–1397.

Baere L, Matheews B, Velghe F (2010), State of the art of anaerobic digestion in Europe in processing of the 12th IWA Anaerobic Digestion world congress ,Guadalajara, Jalisco, Mexico, Nov 3-6 2010.

Bharathiraja B, Yogendran D, Ranjith R K, Chakravarthy M, Palani S (2014), Biofuels from sewage sludge. International Journal of Chem Tech Research, 6(9):4417-4427.

Bilitewski B (2012), The circular economy and its risks. Waste Management, 32(1):1-2.

Calli B, Schoenmaekers K, Vanbroekhoven K, Diels L (2008), Dark fermentative H_2 production from xylose and lactose effects of on-line pH control. International Journal of Hydrogen Energy, 33: 522-530.

Cavinato C, Bolzonella D, Fatone F, Pavan P, Ceechi F (2011), Biohythane production from the organic fraction of municipal solid waste: Improving existing anaerobic digestion plants. International Journal of Hydrogen Energy, 32:2057-2065.

Cavinato C, Giuliano A, Bolzonella D, Pavan P, Cecchi F (2012), Biohythane production from food waste by dark fermentation coupled with anaerobic digestion process: A long-term pilot scale experience. International journal of hydrogen energy, 37: 11549-11555.

Chen CC, Lin CY, Lin MC (2002), Acid–base enrichment enhances anaerobic hydrogen production process. Applied Microbial and Biotechnology, 58: 224–228.

Chinellato G, Cavinato C, Bolzonella D, Heaven S, Banks CG (2013), Biohydrogen production from food waste in batch and semi-continuous conditions: evaluation of a two-phase approach with digestate recirculation for pH control. International Journal of Hydrogen Energy, 38:4351-4360.

Corneli E, Dragoni F, Ragaglini G (2016), Energy conversion of biomass crops and agro industrial residues by combined biohydrogen biomethane systems and anaerobic digestion. Bioresource Technology, 2011:509-518.

Costa JC, Oliveria JV, Abreu AA (2015), Biohythane production from marine macro algae *sargassum sp*. coupling dark fermentation and anaerobic digestion. Bioresource Technology, 190:251-256.

Das D, Verziroglu TN (2001), Hydrogen production by biological processes: a survey of literature. International Journal of Hydrogen Energy, 6:13–28.

Dounavis A.S, Ntaikou I, Lyberatos G, (2015), Production of advanced biobased hydrogen enriched methane from waste glycerol in a two-stage continuous system.

Elreedy A, Tawfik A, Kubota K, Shimada Y, Harada H (2015), Biohythane (H_2+CH_4) production from petrochemical wastewater containing mono-ethylene glycol via stepped anaerobic baffled reactor. International Biodeterioration and Biodegradation, 105: 252-261.

Fabiano B, Perego P (2002), Thermodynamic study and optimization of hydrogen production by *Enterobacter aerogenes*. International Journal of Hydrogen Energy, 27:149–156.

Fang HHP, Li CL, Zhang T (2006), Acidophilic biohydrogen production from rice slurry. International Journal of hydrogen energy, 31:683–92.

Fokkens E (2012), Hydrogen and informatics institute of applied technologies gGmbH.

Gottardo M, Micolucci F, Mattioli A, Faggian S, Cavinato C, Pavan P (2015), Hydrogen and methane production from biowaste and sewage sludge by two phases anaerobic codigestion. Chemical Engineering Transaction, 43:379-384.

Heinimo J, Junginger M (2009), Production and trading of biomass for energy- an overview of the global status. Biomass and Bioenergy, 33(9):1310.

Hung CH, Chang YT, Chang YJ (2011), Roles of microorganisms other than *Clostridium* and *Enterobacter* in anaerobic fermentative biohydrogen production systems. Bioresource Technology, 102:8437–8444.

Kamm B, Gruber PR, Kamm M (2006), Biorefinery industrial process and products. Status and future direction. Wiley – VCH Verlag GmbH & Co. KGaA.

Kasianantham N, Rayapati S, Thangavelu E, Ponnusamy B, Kandasamy A (2011), Hydrogen enriched compressed natural gas- a future fuel for internal combustion engines. Thermal Science, 15(4):1145-1154.

Kavathekar K.P, Rairikar S.D, Thipse S.S (2007), Development of a CNG injection engine complaint to euro-iv norms and development strategy for HCNG operation. SAE paper, 26-29.

Khanal SK, Chen WH, Sung LLS (2004), Biological hydrogen production: effects of pH and intermediate products. International Journal of Hydrogen Energy, 29:1123–1131.

Klell M, Eichlseder H, Sartory M (2012), Mixtures of hydrogen and methane in the internal combustion engine-Synergies, potential and regulations. International Journal of Hydrogen Energy, 37:11531-11540.

Kumari S, Das D (2016), Biologically pretreated sugarcane top as a potential raw material for the enhancement of gaseous energy recovery by two stage biohythane process. Bioresource Technology, 218:1090-1097.

Kvesitadze G, Sadunishvili T, Dudauri T, Metreveli B, Jobava M (2012), Two-stage anaerobic process for bio-hydrogen and bio-methane combined production from biodegradable solid wastes. Energy, 37(1):94-102.

Lin CY, Lay CH (2005), A nutrient formulation for fermentative hydrogen production using anaerobic sewage sludge microflora. International Journal of Hydrogen Energy, 30:285–292

Lin R, Cheng J, Cen K (2016), Enriched energy recovery from cassava ethanol waste water through sequential dark hydrogen, photo hydrogen and methane fermentation combined with ammonium removal. Bioresource Technology, 214:686-691.

Lustrato G, Alfano G, Ranalli G (2012), Bio-hydrogen and bio-methane co-production by sequential two-phase dark fermentation from agro-industrial wastes. Environmental Engineering and Management Journal, 11(3), http://omicron.ch.tuiasi.ro/EEMJ/.

Mabee WE, Gregg DJ, Saddler JN (2005), Assessing bio-refinery sector in Canada. Applied Biochemistry and Biotechnology, 121-124:765-78.

Mariakakis I, Meyer C, Steinmetz H (2012), Fermentative hydrogen production by molasses; effect of hydraulic retention time, organic loading rate and microbial dynamics. Hydrogen Energy-Challenges and Perspectives, Prof. Dragica Minic (Ed.), In Tech, DOI: 10.5772/47750.

Micolucci F, Gottardo M, Bolzonella D, Pavan P (2014), Automatic process control for stable biohythane production in two-phase thermophilic anaerobic digestion of food waste. International Journal of Hydrogen Energy, 39:17563-17572.

Mizuno O, Dinsdale R, Hawkes FR, Hawkes DL, Noike T (2000), Enhancement of hydrogen production from glucose by nitrogen gas sparging. Bioresource Technology, 73:59–65.

Moreno F, Munoz M, Arroyo J, Magen O, Monne C, Suelves I (2012), Efficiency and emission in a vehicle spark ignition engine fueled with hydrogen and methane blends. International Journal of Hydrogen Energy, 37:11495-11503.

Nagendranatha RC, Annie MJ, Naresh K and Venkata SM (2015), Waste remediation integrating with value addition: Bio refinery approach towards sustainable Bio-based technologies. Microbial Factories, 231-256, DOI: 10.1007/978-81-322-2598-0_14.

Noori M, Saady C (2013), Homoacetogenesis during hydrogen production by mixed culture dark fermentation: unresolved challenge. International Journal of Hydrogen Energy, 38:13172-13191.

Pan J, Zhang R, El-Mashad HM, Sun H, Ying Y (2008), Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation. International Journal of Hydrogen Energy, 33:6968–75.

Peixoto G, Filho JLRP, Agnelli JAB, Barboza M, Zaiat M (2012), Hydrogen and methane production, energy recovery, and organic matter removal from effluents in a two-stage fermentative process. Applied Biochemistry and Biotechnology, 168:651–671.

Pisutpaisal N, Nathao C, Sirisukpoka U (2014), Biological hydrogen and methane production from food waste in two stages CSTR, 50:719 – 722.

Porpatham E, Ramesh A, Nagalingam B (2007), Effect of hydrogen addition on the performance of a biogas fuelled spark ignition engine. International Journal of Hydrogen Energy, 32:2057-2065.

Ren NQ, Li JZ, Li BK, Wang Y, Liu SR (2006), Biohydrogen production from molasses by anaerobic fermentation with a pilot scale bioreactor system. International Journal of Hydrogen Energy, 31:2147–57.

Roy S, Das D (2016), Biohythane production from organic waste: present state of art, Environmental Science and Pollution Research (international), 23(10): 9391-410.

Sarkar O, Venkata SM (2016), Deciphering acidogenic process towards biohydrogen, biohythane, and short chain fatty acids production: multi-output optimization strategy. Biofuel Research Journal, 11:458-469.

Seilnacht (http//wwwseilnach.com/lexikon/ebiogas.html).

Si B, Liu Z, Zhang Y, Li J, Shen R, Zhu Z, Xing X (2016), Towards biohythane production from biomass: Influence of operational stage on anaerobic fermentation and microbial community. International Journal of Hydrogen Energy, 41: 4429-4438.

Singhal Y and Singh R (2015), Energy recovery from petha industrial wastewater by anaerobic digestion. International Journal of Science and Engineering, 3:146-151.

Soetaert W, Vandamme EJ (2009), Biofuels in perspective. Biofuels, DOI: 10.1002/9780470754108.ch1.

Sparling R, Risbey D, Poggi-Varaldo HM (1997), Hydrogen production from inhibited anaerobic composters. International Journal of Hydrogen Energy, 22:563–566.

Suraju A, Lateef, Beneragama N, Yamashiro T, Iwasaki M, Umetsu K (2014), Batch anaerobic co-digestion of cow manure and waste milk in two-stage process for hydrogen and methane productions. Bioprocess and Biosystems Engineering, 37:355–363.

Thong SO, Prasertsan P, Intrasungkha N, Dhamwichukorn S, Birkeland NK (2007), Improvement of biohydrogen production and treatment efficiency on palm oil mill effluent with

nutrient supplementation at thermophilic condition using an anaerobic sequencing batch reactor. Enzyme and Microbial Technology, 41:583–590.

Ueno Y, Kawai T, Sato S, Otsuka S, Morimoto M (1995), Biological production of hydrogen from cellulose by natural anaerobic microflora. Journal of Fermentation and Bioengineering, 79:395–397.

Wikipedia: Anaerobic Digestion

www.shell.com

Zhang ZP, Show KY, Tay JH, Liang DT, Lee DJ, Jiang WJ (2006), Effect of hydraulic retention time on biohydrogen production and anaerobic microbial community. Process Biochemistry, 41: 2118–2123.